CHANGES IN THE EPITHELIUM OF THE OVIDUCT OF WHITE MICE AFTER TREATMENT WITH NEUTRAL RED

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The morphological and functional changes of the oviduct in mammals attracted the attention of many authors. A major part of pertinent literary data bear upon the macro- and microscopic morphology of the oviduct of a number of domestic and laboratory animals. These examinations refer to the postnatal development of the oviduct (22), to its periodical construction (1, 8), to the light and electron microscopic structure (10, 24, 31, 35) of the various cell types constituting the epithelium (cilial, secretory, supplementary and peg cells) and to their changes in the individual phases of the sexual cycle (13).

A number of investigations deal with the amount and or cyclic changes of various histochemically demonstrable substances such as enzymes, or components of lipoid and carbohydrate character (3, 6, 9, 10, 12, 14, 32).

On the basis of the results reported so far the construction of the oviduct, and the localization and amount of various histochemically demonstrable substances in this organ can be generally readily defined. It is to be noted, however, that these results do not supply sufficient information on the functional changes of the individual cell types during the sexual cycle. The aim of the present paper is to study this question by using the vital staining procedure.

The theoretical and practical aspects of the application of vital stains were pointed by Möllendorff, Chlopin, Nassonow and Strugger (33, 5, 23, 34). Chlopin (5) has shown that in some cells in the granules containing basic vital dye a substance of basophilic character accumulates after a certain period. These granules which consist of two components, dye and basophilic substance, were named by Chlopin krinom granules. In his opinion krinon granules are protein containing formations, degenerating cells are hardly capable of producing krinom.

Subsequently a number of authors (5, 11, 15, 23) were engaged in investigating the nature and formation of krinom granules. It was found that the dye granules appear in the region of the Golgi apparatus indicating that in this procedure the Golgi apparatus plays a primary role. Kamnyev (15) found

in the krinom granules Feulgen-positive material, that is DNA. Schmidt (29, 30) studying the chemical nature of the krinom granules has established that the dye is bound in the first place to plasma portions rich in RNA and that in these portions, under certain conditions, not only RNA but also DNA may accumulate. In his opinion this accumulation of DNA is a pathological phenomenon.

The procedure of krinom formation was examined in several organs, primarily in the cells of the intestine, liver and pancreas. However no literary data are available on the reactions induced by the stain in the epithelial cells of the oviduct. Morphological and functional changes of the oviduct during the pahses of the sexual cycle prompted us to study the cyclical changes of cells by means of basic stains. The question was how the response of the cells depends on the physiological condition when treating the oviduct with the dye in various phases of the sexual cycle.

Material and method

60 white mice were used in the course of experiments. Before killing them vaginal smear was taken once a day to establish the normal sexual cycle during 2 – 3 weeks. The animals were generally oestrous every 4 – 5 day. Determination of the single phases of the sexual cycle (dioestrus (D_1; D_2; D_3; D_4), prooestrus (P), oestrus (O), metoestrus (M) was carried out by examining vaginal smears according to the method of Odorfer (26).

Animals exhibiting the normal sexual cycle were divided in two groups. The animals belonging to the first group (control) were killed without treatment in the various phases of the cycle. Those of the other group were intraperitoneally injected with neutral red (Neutral red, Chrome); 1 mg/g body weight dissolved in 1 ml distilled water and decapitated 3, 6, 10, 15, 24 hours after the administration of the dye. Before killing the animals the normal progress of the cycle was controlled again by examining the vaginal smears. In the experiment only such animals were used in which, on the basis of repeated vaginal smear examinations, it could be established that the injection of stain had neither inhibiting nor stimulating effect on the cycle.

The oviducts of the one side both of treated and untreated animals, were fixed in formalin – sucrose solution at 4°C. Acid phosphatase activity was demonstrated by incubating 15 – 20 μ frozen sections in a naphtol AS-TR-Fast Garnett GBC mixture. (4).

The oviducts of the other side were fixed in Helly fluid and embedded in paraffin. Then serial sections, of 5 μ thickness, were prepared and stained with 0.1 per cent toluidene blue solution (pH 3.8) suitable to the combined demonstration of both RNA and DNA. Simultaneously PAS reaction was performed.

Experimental results

In the oviduct epithelial cells of the animals treated with the dye (fixation in Helly), dye granules were observed for the first time 6 hours after the administration of neutral red. They appeared at first in the uterinal end cells at

the basal part of the cytoplasm or around the nuclei, but not much later they could be observed in lower amounts also in other parts of the oviduct. After 10 hours it was found that these dye granules had greatly increased and showed a basophilic character. Thus in the epithelial cells of the oviduct of the mouse about 10 to 15 hours from administration of the stain are required for the formation of the basophilic granules. Later on (after 24 hours) a diminution, and discharge of the granules can be observed. In the following the 15 hour material is taken into consideration. The amount of krinom granules developing in the epithelial cells depends on the individual phases of the sexual cycle. Most krinom granules can be observed in the cells during metoestrus and dioestrus; less in oestrus, while they are generally missing in proestrus. In the transitory stages of the cycle (O→M, M→D etc.) a decreased rate of formation of the granules was observed. This cyclic change was equally characteristic of all parts of the oviduct.

Comparing the main parts of the oviduct (ampulla, isthmus, uterinal end) with each other from the point of view of krinom formation it was found that the quantitative fluctuations of krinom granules in the different phases of the cycle showed an identical trend as a rule. In this trend, however, substantial differences in the number, time and localization of the basophilic granules could be observed between the individual parts.

Ampulla

In this part of the oviduct the number of the krinom granules was lowest although it was here that quantitative fluctuations in the phases of the cycle were most conspicuous. Also a time-shift in the formation of the krinom granules was found as compared to the other parts. This is probably due to the later appearance of the dye granules. The basophilic granules in the cells were almost exclusively basally arranged, they were seldom found to occur around nucleus and the cells on the plicae contained more of them than those among the plicae (Fig. 1.).

Isthmus

In the epithelial cells of the isthmus somewhat more basophilic granules developped than in the part near the ovary but this amount was much lower than that at the uterinal end. The krinom granules appeared earlier than in the ampulla and but later than in the uterinal end. The localization of the krinom granules within the cell was different from that at the ends. Though they were found to be arranged in the basal part of the cell, but not in a row but rather in nodes or closely adhering to the nucleus. In these section not the cells of the plicae but those among the plicae contained more krinom granules (Fig. 2.).

Uterinal end

Granule formation induced by the dye was most intensive in the cells of this part. It was here that dye granules appeared for the first time. The size and number of basophilic granules are manyfold not only of those developed in the ampulla but even in the isthmus. The amount of the krinom granules changed according to a cycle similar to the previous parts but did not completely disap-

Fig. 1. Krinom granules in the ampulla, metoestrus.
Helly, toluidene blue (800×)

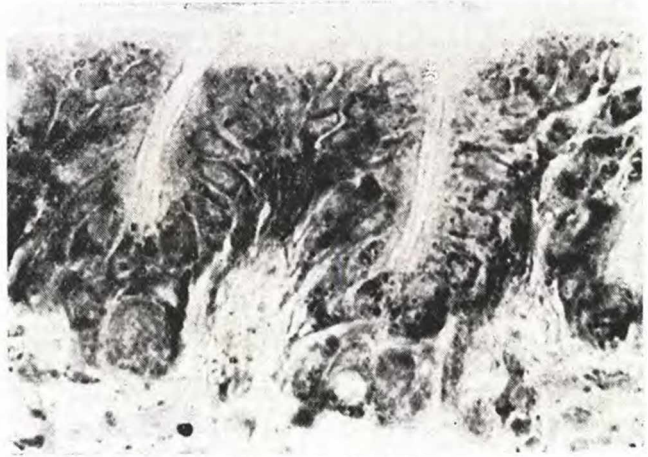

Fig. 2. Krinom granules in the isthmus, metoestrus.
Helly, toluidene blue (800×)

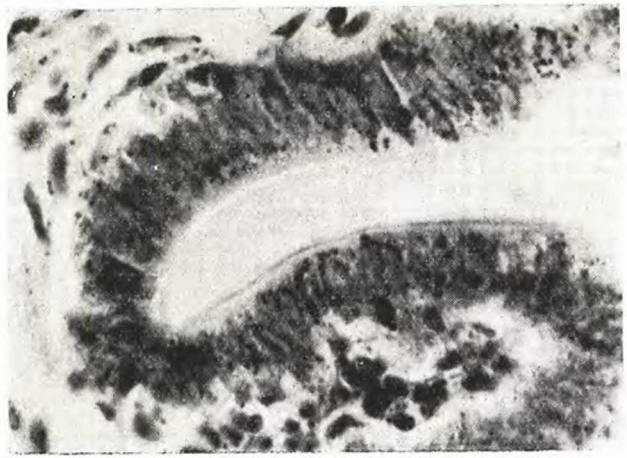

Fig. 3. Krinom granules at the uterinal end, dioestrus.
Helly, toluidene blue (800×)

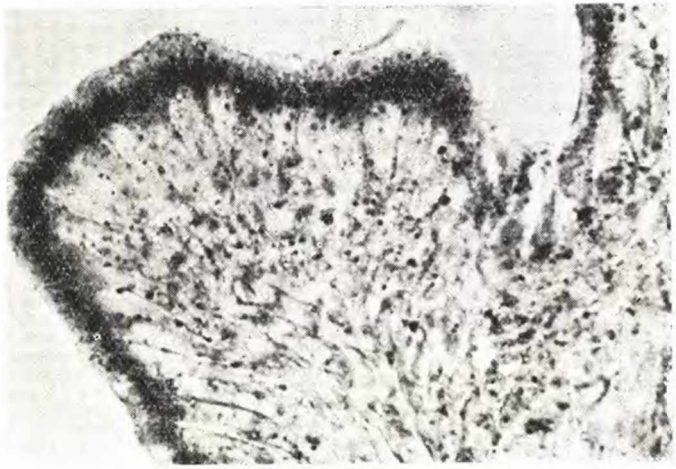

Fig. 4. Uterinal end, dioestrus, control.
Acid phosphatase.

pear even in prooestrus although it was reduced to a minimum. A difference was found also in the localization of the krinom granules within the cells. In the parts dealt with above no substantial differences were found in this respect in the various phases of the cycle. In the case of the uterinal end, however, the krinom granules in dioestrus were for the most part of basal arrangement or they were grouped around the nucleus; in prooestrus they were preferentially found apically while in oestrus and metoestrus in two rows, basally and apically below and above the nuclei. Similarly to the isthmus the krinom granules were found in all phases of the cycle in a higher number in the cells among the plicae (Fig. 3.).

In the epithelial cells of the oviduct of the animals treated with the stain a great amount of PAS positive granules could be demonstrated. The localization of these in the cell was similar to that of the krinom granules. The PAS positive granules similarly to the basophilic granules appeared first of all at the uterinal end and were found here in the greatest amount while they appeared later in the ampulla and their number was invariably less. As a rule their localization in the epithelial cells was similarly basal, and they were arranged in rows or nodes and around the nucleus. Only in the uterinal part were there found in addition to basally arranged granules also granules of apical arrangement. In the sections where there were large, high plicae (ampulla) the majority of the PAS-positive granules were found in the cells of the mucous membrane covering the plicae whereas in the sections with lower plicae (isthmus and uterinal end) the epithelial cells in the lower part of the plicae contained more of them. Quantitative fluctuation of the PAS-positive granules during the sexual cycle also agreeds with the changes of the basophilic granules. Their number was smallest in prooestrus and increased very rapidly in the inactive phases. From all this it may be concluded that the PAS-positive granules and the krinom granules are identical, that is the latter, in addition to the basophilic material contain also PAS-positive substance.

As far as the activity of acid phosphatase in the oviduct of control animals is concerned it was found that the cytoplasm of the epithelial cells showed great activity of acid phosphatase localized in fine granules. The intensity of the reaction showed, however, substantial differences in the various phases of the sexual cycle and in the individual parts of the oviduct. Highest acid phosphatase activity was observed during dioestrus while lowest in prooestrus.

In the distribution of acid phosphatase granules, in their localization within the cell and in their changes with respect to cycle substantial differences appeared between the parts.

Highest activity was demonstrated invariably in the epithelial layer of the uterinal end, in the apical part of the cells (Fig. 4.). The activity of acid phosphatase was always high in this section although a substantial reduction could be observed in prooestrus.

In the isthmus the activity of acid phosphatase was generally lower then in the uterinal end. A substantial reduction of activity could be observed here not only in the proestrus but also during the oestrus. In the cells the granules exhibiting acid phoosphatase activity appeared in the area just below or above the nucleus. The lowest activity of acid phosphatase was observed in the epithelial

layer of the ampulla. Here the granules giving positive reaction were arranged just below the nucleus and in conformity with the cycle their amount dropped to the minimum in prooestrus and early in oestrus while at the end of the oestrus and in metoestrus gradual increase of phosphatase activity was observed.

Under the influence of the dye acid phosphatase activity changed in every part of the oviduct. These changes were most definite with the animals decapitated 15 hours after the administration of the stain. In the ampulla simultaneously with the development of the krinom granules the acid phosphatase activity disappeared or only an insignificant amount could be demonstrated, that is the development of the krinom granules resulted in an almost complete less of acid phosphatase activity (Fig. 5.).

Fig. 5. Ampulla, dioestrus, acid phosphatase. *a)* Control, *b)* treated with neutral red

In the epithelial layer of the isthmus under the influence of the stain the reduction of acid phosphatase activity was also of a high degree but its complete absence was not observed (Fig. 6.).

The least change under the influence of the stain was found in the epithelial layer of the uterinal end but the activity of acid phospahatase exhibited also in this section a substantial reduction.

The results obtained revealed that the development of krinom granules influenced acid phosphatase activity in the epithelial cell of each part of the oviduct. Simultaneously with the development of the krinom granules acid phosphatase activity was greatly reduced of disappeared completely.

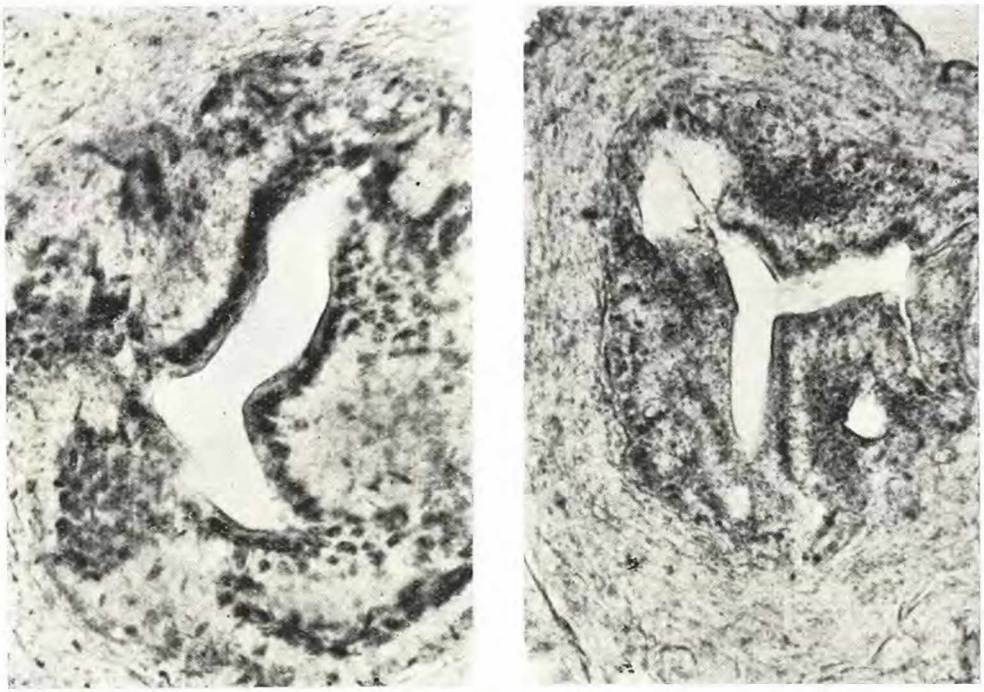

Fig. 6. Isthmus, dioestrus, acid phosphatase. *a)* Control, *b)* treated with neutral red

Discussion

Our examinations have demonstrated that the epithelial cells of the oviduct similarly to other cells are able to store the neutral-red absorbed in the form of granules and to transform it into granules of the basophilic type. Granule development is in close connection with the sexual cycle, that is it depends upon the physiological condition of the cell. The strongly increased or intensively secerning cells in a more active state were less able to develop krinom granules than the inactive ones (degenerating peg and club shaped cells, ciliar and light cell).

On the basis of earlier investigations several authors have assumed that granule formation occurs in the Golgi-apparatus (5, 11, 15, 23). According to our experiences the granule formation, taking place in the epithelial cells of the oviduct, contradicts this assumption. The krinom granules in the epithelium of the oviduct are for the most part basally arranged, less of them are arranged around the nuclei and only an insignificant amount can be found in the upper part of the cells in the region of the Golgi apparatus. Besides, in the secretory cells less krinom granules are formed than in the other cells although the Golgi apparatus is more developed in the secretory and generally in the intensively active cells than in those of reduced activity or in aged cells. In the large, light cells of the epithelium which according to B a l b o n i

(3) are exhausted secretory cells, intensive granule formation takes place. On the basis of all these it does not seem probable that in the cells of the oviduct the dye absorbed would concentrate in the Golgi apparatus or that it would have a decisive role in granule formation.

According to more recent data rather the lysosomes seem to be involved in granule formation (19, 20, 21, 27, 28, 29, 30, 2). It is a well known fact that lysosomes are able to take up and store various foreign substances (7, 25). It may be assumed that stain which gets into the cell, similary to other foreign substances that entered the cytoplasm is also concentrated in the lysosomes where basophilic substances accumulate too. Acid phosphatase activity and the presence of PAS-positive substances are equally characteristic of lysosomes. Our present experiments also demonstrate that the lysosomes have a role in the formation of krinom granules since under the influence of stain the acid phosphatase content is greatly reduced and similary to the lysosomes the krinom ganules also contain PAS-positive substance. It is also remarkable that there is a parallelism between the krinom forming capacity of the cells and the acid phosphatase activity, i.e. the change in both is identical. In the ampulla and in the isthmus the localization of acid phosphatase is similar to that of the krinom granules. On this basis it may be concluded that not so much the Golgi apparatus but rather the lysosomes are involved in granule formation. Krinom granules develop mostly in those phases of the sexual cycle (metoestrus and dioestrus) in which acid phosphatase activity is most intensive.

Summary

Epithelial cells of the oviduct accumulate neutral red in granular form and in these granules a substance of basophilic type (Chlopin's krinom) appears. The dye binding capacity of the epithelial cells of the oviduct depends on the phases of the sexual cycle. Krinom granule formation is more intensive in the inactive phases of the sexual cycle than in the more active ones; that is, krinom granules reach the largest amount in metoestrus and dioestrus; in oestrus their number is insignificant and they are not found in prooestrus. The period of the formation of the krinom granules, their localization within the cell and their amount in the various parts of the oviduct is different. In the epithelial cells of the anpulla the number of krinom granules developed is lowest as compared to the other parts, their formation sets in later, they are almost exclusively of basal arrangement and the quantitative fluctuation observed in the phases of the sexual cycles is most conspicuous in this part. On the other hand, in the isthmus somewhat more krinom granules develop, they appear sooner, they are arranged basally in nodes rather than rows. In the epithelium of the uterinal end granule formation is most intensive. In this section krinom granules appear for the first time and develop to the highest amount, they are found both basally and apically and although the quantitative fluctuation observed in the phases of the cycle appears here too, it is of a lower degree than in the ampulla or isthmus sections.

Acid phosphatase activity is different in the various phases of the sexual cycle. It is highest in metoestrus and dioestrus when also krinom formation is

most intensive while in prooestrus in all parts of the oviduct the activity is minimal or can not be demonstrated at all. Acid phosphatase activity simultaneously with the development of the krinom granules greatly diminishes or is absent. According to our data it can be assumed that lysosomes have a role in the accumulation of stain and in the development of krinom esgranules.

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